

Macrophage Response to UHMWPE Submitted to Accelerated Ageing in Hydrogen Peroxide

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Abstract – Ultra-high molecular weight polyethylene (UHMWPE) slices were submitted to accelerated oxidation in hydrogen peroxide 30% (vol%) solution for different periods of time. Then, the oxidative degradation of samples was characterized by FTIR and the inflammatory response was assessed by macrophage nitric oxide secretion comparing oxidized UHMWPE to the original polymer. The results have given evidence that the accelerated aging of UHMWPE was achieved by oxidative degradation and it was higher for longer periods of test. Moreover, the macrophage response has shown a good correlation with the oxidation degree of UHMWPE.

In the present study, an accelerated ageing by oxidative degradation of UHMWPE in hydrogen peroxide solution was performed and the macrophages response by nitric oxide's secretion was analyzed in order to verify the influence of chemical surfaces in the inflammatory process. UHMWPE used for orthopaedic prosthesis can be oxidized during sterilization processes, such as γ -ray irradiation; it has been suggested that this alteration is the first step in the prosthesis aseptic loosening process [1]. Experiments were conducted with slices of UHMWPE with thickness of 150-250 μ m. Non-oxidized slices triplicates (n=3) of UHMWPE were immersed in hydrogen peroxide 30 v/v% and incubated at 37°C. The accelerated aging was monitored until 120 days by Fourier Transformed Infrared Spectroscopy (FTIR). Spectra were collected in transmission mode and the total level of oxidation (I_{ox}) was according to ISO 5834-2.

The murine macrophage cells (C57BL6) were cultured in RPMI at 37°C in a humidified incubator with 5% CO₂. Slices of UHMWPE and ox-UHMWPE (oxidized) were placed into microplates (24-wells). The positive control group was cells with lipopolysaccharide endotoxin (LPS) and interferon gamma (IFN- γ). Cells were plated at 1×10^5 density on the wells and incubated at 37°C for 48h. The nitric oxide secretion was evaluated using a scanning multi-well spectrophotometer (ELISA plate reader). The morphology of cells was assessed by Scanning Electron Microscopy (SEM). Before microscopy analysis, specimens were fixed with 2% glutaraldehyde aqueous solution and coated with a thin layer of gold to make them conductive using low deposition rate.

The main peak in the FTIR spectra (not shown) in the 1,700 – 1,750 cm⁻¹ region, corresponding to the strong signal of carbonyl (C=O) groups, has shown a significant increase on absorbance in the test period from 7 up to 120 days. These findings suggested that UHMWPE oxidation has taken place via chemical reactions of the polyethylene chain with hydrogen peroxide aging solution. Furthermore, the nitric oxide secretion has been higher for ox-UHMWPE 120 days compared to UHMWPE, Fig. 1. The SEM image (Fig.2) has clearly shown the presence of macrophage. Also, the overall shape and the presence of pseudopodia observed for the peritoneal macrophage cultures are in good agreement with reported literature [2]. In conclusion, the results provided strong evidence that macrophage response was specifically correlated to extension of chemical degradation of UHMWPE surface.

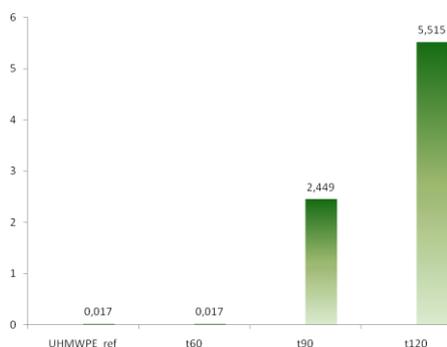


Figure 1: (a) Nitric oxide secretion of macrophage; UHMWPE-ref (original); t60 (ox-UHMWPE 60 days); t90 (ox-UHMWPE 60 days); t120 (ox-UHMWPE 120 days).

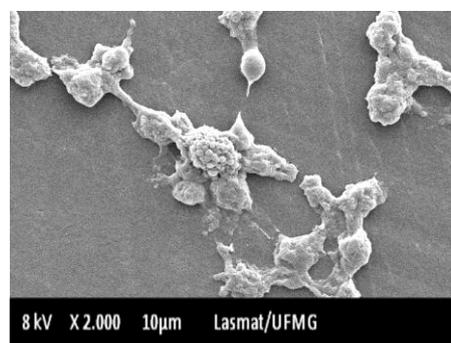


Figure 2: SEM image of morphology macrophages in contact with oxidized surface of UHMWPE for 120 days (2,000X magnification).

[1] F. Renó, F. Lombardi, and M. Cannas, *Biomaterials*. 24 (2003) 2895-2900.

[2] M. Bosetti, L.Zanardi, P. Bracco, L. Costa, and M. Cannas, *Biomaterials*. 24 (2003) 1419-1426.